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THE MORPHOLOGY OF SPOROTHRIX SCHENCKII IN TISSUES AND IN ARTIFICIAL MEDIA.*

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A point of some interest in connection with the study of fungi of various kinds is the difference that appears in the morphology of the organisms when growing in the living tissues and when growing on artificial media. The variations, for example, of actinomyces and blastomyces in this respect are well-known phenomena. Similar observations on sporothrices have also been observed and it is the purpose of this paper to inquire further into this matter with a view to determining under what conditions such variations in morphology occur.

In living tissues sporothrix organisms always appear as elongated or oval bodies fairly uniform in size and often showing distinct budding processes (Fig. 1). The arrangement is usually single, but frequently two or more are found end to end, and at times several may be arranged radially about a central spore. In experimental sporotrichosis as a rule these forms are numerous in the tissues and easily stained. In the human body they may at times be present in large numbers but usually they are so few in number that it is difficult or impossible to find them. For convenience I shall call these forms, "tissue forms."

Branched mycelial filaments are not found in the living tissues either in the human or the experimental disease. These mycelial threads or hyphae develop abundantly on practically all the artificial media that have been used and at their sides and extremities form numerous spores, oval in shape and differing decidedly in appearance from the tissue forms. In addition to the filaments and spores, typical tissue forms may also be seen in small numbers in artificial culture, especially in fluid media, and may present budding processes like those in tissues.

Let us first compare the method of germination of mycelium

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from spores, with the process of budding as observed in the tissue forms. In the former a bulging may be seen on one side of the spore looking at first not unlike a pseudopod. This increases and either extends in one direction, in which case the original spore will appear at the extremity of the filament or, as often happens, the pseudopod-like structure immediately divides into two branches proceeding in opposite directions, in which case the original spore appears at the side of the filament. Not infrequently, too, the filaments may arise at opposite sides of the spore or several may thus arise. The formation of buds in tissue forms, on the other hand, occurs as a very minute protrusion of protoplasm from the end of the organism and never from the side. The daughter cell at first is very small but soon increases in size, the pedicle remaining very fine and delicate. This process is quite comparable to the formation of spores at the ends and along the sides of the filaments, but it is evidently quite different from the formation of mycelial threads. The factors which control the formation of the one or the other apparently have not been investigated, nor do we know the conditions of their development.

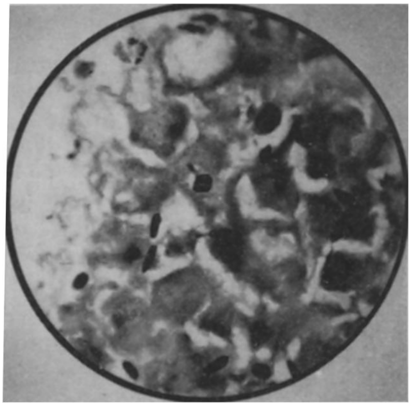

FIG. 1.—Spindle forms of sporothrix in pus from an infected mouse. Note the budding processes. ×1,200.

In order to investigate this point the cultivation of sporothrices in various media and under various conditions was made. It was found that when the sporothrix is cultivated in certain body fluids, the tendency to form branching filaments is markedly diminished and in some instances quite absent, whereas in general the tendency to develop tissue forms is decidedly increased. In urine, joint fluid, cerebrospinal fluid, and ascites fluid, the sporothrix thrives, forming a growth which after a few days settles to the bottom of the tube, the fluid above remaining quite clear. In all the above media not only are branching filaments with many spores seen, but also some tissue forms with budding processes may be found. When the sporothrix is grown in defibrinated blood, however, the number of tissue forms as a rule greatly predominates, in some tubes scarcely a filament occurring. In washed blood and in serum the same is true, though possibly not to the same extent. Heated and unheated sheep serum, human serum, and dog serum all give about the same result, there being no constant or appreciable differences. Distinct growth takes place in all with the occurrence of many typical tissue forms, the filaments being infrequent. In defibrinated blood after growth of about two weeks it is common to see groups of the budding tissue forms arranged radially in large clusters of 20 to 30 or even 50 or more organisms around a central spore or very short filament and simulating, in a measure, actinomyces (Fig. 2).

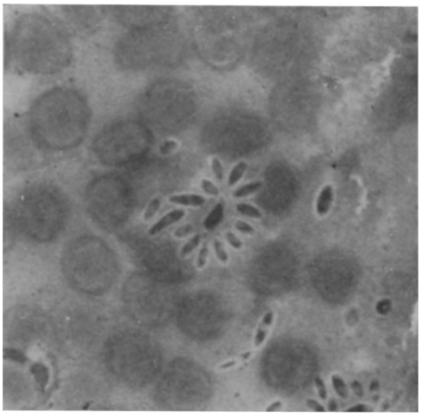

FIG. 2.—Sporothrix growing in pure blood *in vitro*. Note the spindle forms arranged in ray-like clusters. ×1,200.

Growth of sporothrix in the serum and blood of rabbits which were immunized against the same organism and whose serum readily agglutinated various strains of sporothrix,[1] did not appear as abundant as in normal fluids. However, some growth did occur which in microscopic appearance was not essentially different from that occurring in normal fluids, there being both tissue forms and some filaments present.

When cultures in blood are grown under paraffin oil, similar or even better results are obtained, there being usually little else than tissue forms present. This is also true, but to a less extent, of broth cultures when grown under oil, a fact which would suggest that the partial anaerobic conditions play a rôle in determining the morphology of the organism. The sporothrix is strictly aerobic. If a very small amount of oxygen is permitted to enter a tube of inoculated glucose agar, proliferation occurs with the formation of some hyphae but also with the production of a large number of the elongated forms quite like the tissue forms. It may be pointed out here that a somewhat similar observation has been made by Hamburger[1] in connection with blastomyces, in which case certain involution forms appear in anaerobic, glucose agar cultures after several days.

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[1] *Jour. Infect. Dis.* 1913, 12, p. 140.

Further observations were made *in vitro* on the growth of sporothrix when in contact with sterile animal tissues. Such tissues including spleen, liver, and kidney, when placed in test tubes and partially immersed in serum, form an excellent medium for this organism, the growth after a few days covering the surface as a grayish soft mat. Smears from the surface show both mycelial branching forms and tissue forms. On examining stained sections of these tissues after 10 days or two weeks one sees that the sporothrix has already invaded the tissues to a considerable extent. Directly on the surface may be seen some mycelia but in the dead tissues beneath, there occur in enormous numbers, only the typical elongated tissue forms (Fig. 3) such as appear in the human and the experimental disease. Here branching filaments are never seen. It is an interesting fact that in agar cultures, for instance, branching filaments readily penetrate the subjacent agar in all directions, but they are not able to penetrate either living or dead tissues under similar conditions. It therefore appears that, artificially by using blood or dead sterile tissue under aerobic conditions, we can obtain forms of sporothrix practically identical with those occurring in living tissues.

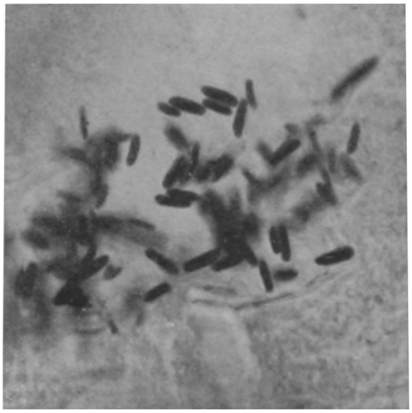

FIG. 3.—Spindle forms of sporothrix growing in sterile kidney tissue *in vitro*. ×1,200.

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[1] *Jour. Infect. Dis.*, 1907, 4, p. 201.

Reference may here be made to a strain of sporothrix isolated and described by Splendore and referred to by Gougerot[1] as *Sporothrix asteroides.* This organism is characterized by the formation, *in vivo* only, of star-like structures or asteroid forms consisting of a central body with radially arranged, elongated spores. Inasmuch as raylike forms of ordinary sporothrices are commonly seen in tissues, it is not unlikely that in this strain the tendency to form such asteroid clusters is more marked than usual and that they are probably not distinctive structures in themselves.

These facts remind one somewhat of the behavior of the ray forms of actinomyces in tissues. The club forms of this organism which may be roughly compared with, though probably are not analagous to, the "tissue forms" of sporothrix, do not occur on artificial media unless, as has been observed by Wright, body fluids, such as blood serum or pleuritic fluid, are added. Wright regards the club formation as a process designed to protect the organism from the tissue reactions. Others regard the clubs as degeneration forms and still others as organs of fructification. Whatever may be their function the similarity in form of sporothrix, when grown in blood, to the ray fungus is at times rather striking, a fact which may be associated with the observation made by Widal and Abrami[2] that a group relationship based upon serum tests exists between sporothrix and actinomyces.

The observations recorded in this paper concern various strains of sporothrix including a strain isolated in this country by Hektoen in 1898 and named by him *Sporothrix Schenckii,* and also a strain obtained from Dr. Gougerot of Paris and there known as *Sporothrix Beurmanni.* It may be stated that no essential differences in behavior were noted between the various strains when grown in the different media and under the conditions as detailed above.

SUMMARY.

In human and experimental sporotrichosis the sporothrix always appears as oval or elongated bodies and never forms distinct hyphae.

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[1] Kolle and Wassermann, *Handbuch der Path. Microorg.*, Zweite Auflage, Bd. V, p. 225.

[2] *Ann. de l' Inst. Pasteur,* 1910, 24, p. 1.

On ordinary artificial culture media, long branching hyphae appear together with some morphological forms like those found in tissues.

Sporothrix grows readily in various body fluids. In some of these fluids, especially blood, the tissue forms may greatly predominate.

Sterile animal tissues *in vitro* furnish an excellent medium for growing sporothrix and here the tissue forms appear exclusively.

Partial anaerobic conditions seem to play a rôle in causing these morphological variations though there are probably other determining factors.